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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): Howard Milne Chandler  
U.S. National Phase of PCT/AU99/00310  
International Filing Date: April 27, 1999  
Title: SAMPLE COLLECTION METHOD  
Docket No.: 0141-2004

RECEIVED

14 MAY 2001

Legal Staff  
International Division

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Date of Deposit with U.S. Postal Service: 10/27/00

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Tammy L. Moulton

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Tammy L. Moulton

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U.S. NATIONAL STAGE  
PATENT APPLICATION TRANSMITTAL

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Dear Sirs:

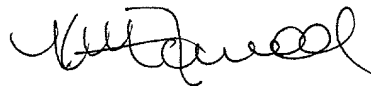
Pursuant to the requirements specified in 37 CFR 1.495(b), transmitted herewith is a copy of the above-referenced International Application.

Please charge Deposit Account No. 06-0130 in the amount of \$1,000.00, the basic national fee as required by §1.492. Any deficiency or overpayment should be charged or credited to Deposit Account No. 06-0130.

Applicant respectfully requests examination of the subject application as to the patentability of the invention in the United States of America.

Please direct all correspondence to Farrell & Associates, P.C., P.O. Box 999, York Harbor, ME 03911.

Respectfully submitted,



Kevin M. Farrell  
Attorney for Applicant(s)  
Registration No. 35,505

York Harbor, ME

Dated: 27-07-00

## SAMPLE COLLECTION METHOD

### FIELD OF THE INVENTION

This invention relates to a method for collecting a sample for subsequent use  
5 in the detection of an analyte in the sample. In one particular embodiment, this invention relates to a method for sampling faecal material for the purposes of subsequent detection in the sample of occult blood or one or more other indicators of a pathological condition.

10 The present invention also extends to an assay kit which is particularly suitable for the purposes of detection in a sample derived from faecal material of occult blood or one or more other indicators of a pathological condition.

### BACKGROUND OF THE INVENTION

15 A well known and widely-used clinical reagent for the detection of occult blood in a sample, particularly a faecal sample, is guaiac (also known as gum guaiac or resin guaiac). When used in association with an appropriate developer solution, guaiac provides a colorimetric assay system for detecting haemoglobin in the sample. Such tests are commercially available, for example, Hemoccult II and Hemoccult II Sensa  
20 (SmithKline Diagnostics, San Jose, California, USA).

Prior Australian Patent No. 665956 (International Patent Application No. PCT/US92/04425) notes that among the many analytical systems used for detection and/or determination of analytes, particularly analytes of biological interest, are  
25 chromatographic assay systems. Among the analytes of biological interest frequently assayed with such systems are:

1. hormones, such as human chorionic gonadotropin (hCG), frequently assayed as a marker of human pregnancy;
2. antigens, particularly antigens specific to bacterial, viral, and protozoan  
30 pathogens, such as *Streptococcus*, hepatitis virus, and *Giardia*;

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3. antibodies, particularly antibodies induced as a result of infection with pathogens, such as antibody to the bacterium *Helicobacter pylori* and to human immunodeficiency virus (HIV);
4. other proteins, such as haemoglobin, frequently assayed in determinations of faecal occult blood, an early indicator of gastrointestinal disorders such as colon cancer;
5. enzymes, such as aspartate aminotransferase, lactate dehydrogenase, alkaline phosphatase, and glutamate dehydrogenase, frequently assayed as indicators of physiological function and tissue damage;
6. drugs, both therapeutic drugs, such as antibiotics, tranquillisers and anticonvulsants, and illegal drugs of abuse, such as cocaine, heroin, and marijuana; and
7. vitamins.

Such chromatographic systems are frequently used by physicians and medical technicians for rapid in-office diagnosis and therapeutic monitoring of a variety of conditions and disorders. They are also increasingly used by patients themselves for at-home monitoring of such conditions and disorders.

Among the most important of such chromatographic systems are the "thin layer" membrane-based systems in which a solvent moves as a solvent front across a thin, flat absorbent medium (e.g., nitrocellulose membrane). Among the most important of tests that can be performed with such thin layer systems are immunoassays, which depend on the specific interaction between an antigen or hapten and a corresponding antibody. The use of immunoassays as a means of testing for the presence and/or amount of clinically important molecules has been known for some time.

Chromatographic techniques used in conjunction with immunoassays include a procedure known as immunochromatography. In general, this technique uses a disclosing reagent or particle that has been linked to an antibody to the analyte to be

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assayed, forming a conjugate. This conjugate is then mixed with a specimen and, if the analyte to be assayed is present in the specimen, the disclosing reagent-linked antibodies bind to the analyte to be assayed, thereby giving an indication that the analyte to be assayed is present. The disclosing reagent or particle can be identifiable  
5 by colour, magnetic properties, radioactivity, specific reactivity with another molecule, or another physical or chemical property. The specific reactions that are employed vary with the nature of the analyte being assayed and the sample to be tested.

The present invention is particularly, but not exclusively, directed to collection  
10 of samples derived from faecal material for occult blood detection, for example in screening for colorectal cancer. As previously described, guaiac testing provides a colorimetric assay system for detection of haemoglobin in a sample, however because of the large number of false positives obtained in guaiac testing, in screening programs the use of two or three guaiac tests has been recommended, confirmed when positive  
15 by an immunological test for human haemoglobin (Favennic L., Kapel N., Meillet D., Chochillon C. and Gobert J.G., *Annales de Biologie Clinique*, **50**(5):311-3, 1992). More recently, a combination of guaiac and immunological testing has been suggested (Allison, J.E., Tekawa, I.S., Ransom, L.J. and Adrian, L.L. *N. Engl. J. Med.*, **334**:155-9, 1996).

20

It is an object of the present invention to provide a sample collection method which is simple and economic, and which enables subsequent detection and/or determination of analyte in the sample to be readily carried out, for example using a guaiac test, and/or an immunochromatographic or other immunodiagnostic procedure.

25

#### SUMMARY OF THE INVENTION

In accordance with the present invention, there is provided a method for collecting a sample derived from faecal material, comprising contacting the faecal material with a fluid and subsequently collecting a sample of the fluid with a brush or

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brush-like device having flexible or semi-flexible bristles, wherein the sample of the fluid is collected within the bristles of the brush or brush-like device.

Preferably, the fluid is water.

5

The term "brush" as used herein is used to denote device comprising a stem or handle, usually elongate, and a clump, bunch or group of bristles, hair or other similar flexible or semi-flexible elongate strands, laminar flaps or the like attached to the stem or handle. The term "brush-like device" is used herein to denote a device which is  
10 similar to a brush in that it includes a bunch, clump or group of bristles, hair or other similar flexible or semi-flexible elongate strands, laminar flaps or the like. Whilst reference is made throughout the present specification to the collection of a sample within the bristles of a brush or brush-like device, it is to be understood that the reference to "bristles" is used to include the hairs or other similar flexible or semi-  
15 flexible elongate strands, laminar flaps or the like of a brush or brush-like device.

Preferably, the bristles of the brush or brush-like device will have a length of about 0.2 to 3 cm long, more preferably a length of 1 to 2 cm.

20 In another embodiment, the present invention also extends to an assay kit for testing faecal material which comprises a sample collection device which is a brush or brush-like device having flexible or semi-flexible bristles, together with means for detection of an analyte in a sample derived from faecal material.

25 Such an assay kit is particularly suited for use in detection of occult blood in a sample derived from faecal material. The detection of occult gastrointestinal bleeding is a common method for screening for colorectal cancer. Commonly referred to as the faecal occult blood (FOB) test, a variety of formats are known in the art (see, for example, US Patent Nos. 3996006; 4225557; 4789629; 5064766; 5100619;  
30 5106582; 5171528; 5171529; and 5182191). The majority of test formats are based

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on the chemical detection of the heme groups present in faecal material as a breakdown product of blood. In such tests, the pseudoperoxidase nature of the heme group is used to catalyse a colorimetric reaction between an indicator dye and peroxide. The oxygen sensitive dye can be gum guaiac, orthodianisidine, 5 tetramethylbenzidine, or the like, with guaiac being preferred.

The means for detection of an analyte in a sample which is incorporated into an assay kit as described above may, for example, be means for carrying out a guaiac test for the detection of occult blood in the sample. Alternatively, or additionally, the 10 means for detection of an analyte in a sample may be means for detection of occult blood (or other diagnostic antigens) in the sample by means of a chromatographic procedure, particularly by an immunochromatographic or other immunodiagnostic procedure which is well known in the art. Suitable immunochromatographic procedures are described, by way of example, in US Patent Nos. 5591645 and 15 5622871, the disclosures of which are incorporated herein by reference.

Whilst the present invention is particularly useful in FOB testing as described in detail herein, it is to be understood that the method and assay kit as broadly described herein may be used in sampling faecal material and subsequent testing of 20 the sample to detect the presence of one or more other indicators of a pathological condition, for example, tumour-derived antigens, in addition to or instead of FOB testing.

Throughout this specification, unless the context requires otherwise, the word 25 "comprise", and or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

## DETAILED DESCRIPTION OF THE INVENTION

In the most preferred embodiment, the present invention relates to the use of a brush as a device for obtaining a sample derived from faecal material, and particularly stool, in a fluid such as water, particularly for the detection of occult blood as an indicator of colorectal cancer (CRC) or its precursor conditions.

Most existing faecal occult blood tests (FOBTs) use a sampling stick or paddle to take smears directly from the surface of a collected faecal sample. European Patent Application No. EP 0 727653 discloses the use of a brush device having stiff bristles to collect a sample from the surface of faecal material directly on the bristles. Many CRCs or their precursors (e.g. adenomas > 1cm), bleed into the lumen of the small intestine. As these malignancies arise as protrusions from the wall of the intestine they make contact with the surface of the stool in their region of contact as the stool passes that point. The blood, therefore, may not be evenly distributed through or over the stool. As a result, existing tests that rely on surface sampling of the stool may or may not sample from that portion of the stool where blood is present.

If the stool or other faecal material is sampled in a fluid, for example, when it is in the water of the toilet bowl, there is a better opportunity to gain a representative sampling of the whole stool. This is particularly the case where a small brush (e.g. a small artist's paint brush having bristles about 1 to 2 cm in length) is used for sampling. A brush may be used to "paint" the surface of the stool so as to displace any blood on the surface of the stool into the water surrounding the stool. The flexible or semi-flexible bristles of the brush will be relatively "open" during this brushing and sampling process, but will "close" as the brush is withdrawn from the water, thereby keeping a sample of the water (and any blood contained therein), surrounding the stool within the interstitial spaces of the bristles. This sample may then be transferred to a suitable assay device for subsequent testing.

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By way of contrast, if an absorbent sampling device, such as a swab, was used for sampling, water would infiltrate the fibre windings of the swab on its first contact with the water in the toilet bowl. In this case, there would be little chance of effective displacement of the infiltrated water by any blood-containing water in the vicinity of the stool, and as a result the sampling procedure would not effectively sample any such blood-containing water.

Alternatively, if a solid sampling device such as a solid sampling stick or paddle, or a loop or barbed probe was used, the water sampled from around the stool would be lost as the device was withdrawn through the water of the bowl, and once again the sampling procedure would not effectively sample any blood-containing water.

A further advantage which is obtained by the use of a brush or brush-like sampling device in accordance with the present invention is that the fluid sample collected within the bristles of the sampling device as described above is collected in a semi-quantitative manner, in that the amount of fluid held within the interstitial spaces of the bristles of the sampling device will be a reasonably constant amount for any particular size and configuration of the sampling device.

As described above, an important feature of the sampling device is that the bristles of the device, as defined above, are flexible or semi-flexible. This enables the device to be used to obtain a sample of fluid surrounding the faecal material into which any occult blood on or at the surface of the faecal material has been dispersed, instead of attempting to obtain a sample directly from the surface of the faecal material where it may only be present in isolated locations, and accordingly where there is a risk that any sample taken directly from the surface of the faecal material may not be taken from a location where any blood is present.

As previously described, colorectal cancers and adenomas often bleed into the lumen of the large bowel. Initially, only a small, localised amount of blood leakage may

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occur, leading to isolated spots or areas of blood occurring on the surface of faecal material in the large bowel which will be exposed to the blood first. It is not unreasonable to assume that much of this blood will remain on the surface of this faecal material after it is passed. Similarly, almost all colorectal cancers and all  
5 adenomas occupy only a small portion of the diameter of the large bowel. Therefore, it is also likely that the blood from such lesions will be striped along the faecal material. If this is the case, the brush method of the present invention for sampling faecal material will have an advantage over traditional FOBT sampling methods because the sampling method of the present invention takes a more representative sample than  
10 that of the traditional methods.

Further features of the present invention are more fully described in the following Example(s). It is to be understood, however, that this detailed description is included solely for the purposes of exemplifying the present invention, and should not  
15 be understood in any way as a restriction on the broad description of the invention as set out above.

### EXAMPLE 1

20 The suitability of a brush for sampling blood in water has been shown to be effective by several means:

1. Blood (10  $\mu$ L) was added to water (50 mL) in a beaker. After the blood settled  
25 to a discrete drop at the bottom of the beaker, a brush (#5, LiFung, Hong Kong) was first used to sample the surface water from the beaker. This sample tested negative in a faecal occult blood (FOB) test (Enterix). After mixing the contents of the beaker, a second, similar brush was shown to be capable of selectively sampling sufficient of the blood to be detected in a similar FOB test.

30

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2. A stool sample was injected with blood (50  $\mu$ L) so that the blood was sequestered within a crevice in the stool. The stool was added to a toilet bowl and brushes as described above were used to sample:

- 5 (a) The water of the bowl.
- (b) The water surrounding the stool after the surface of the stool was "brushed".

10 When tested in FOB tests (Enterix), samples (a) tested negative for blood, whereas samples (b) tested positive. In this experiment it may be expected that the sequestered blood would have been missed by conventional sampling of the stool surface with a stick or paddle.

- 15 3. Table 1 below shows the results of a series of experiments to test the effectiveness of sampling of stool samples with a brush as described above. Blood was added directly to normal stool samples, before or after the deposition of the stools into a toilet bowl. Normal stools and the bowl water before stool addition were also sampled. In each case samples collected by the brush
- 20 method were tested for the presence of blood by an FOB test (Enterix).

TABLE 1

FOB Test Results	Bowl Water	Normal stool (i.e. no addition)	25 $\mu$ L blood added	50 $\mu$ L blood added	100 $\mu$ L blood added
No. positive	-	-	4/4	15/15	27/27
No. negative	2/2	15/15	-	-	-

As shown in the Table, all toilet bowl water and normal stool samples tested

5 negative in the FOB test, whereas all samples with added blood ( $\geq 25$   $\mu$ L) gave a positive test result. These results compare favourably with the sensitivity and

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specificity data reported with tests that use direct stool sampling with a sampling stick (Rosen, P., Knaai, J. and Samuel, Z. *Dig. Dis. Sci.*, **42**(10):2064-71, 1997).

## EXAMPLE 2

5

The aim of this study was to determine if the sampling method of the present invention is more capable of detecting significant quantities of blood than a traditional method of FOBT sampling when the blood is striped along one side of the surface of a stool.

10

### Methods

Ten faecal samples were collected from three individuals and spiked with blood to a concentration of 0.5 milligrams of haemoglobin per gram faeces. Spiking was achieved by spotting the blood along the surface of the stool in a stripe.

15

Five spiked stools were tested both by the method of the present invention (EnterixOBT) and by FlexSureOBT (Beckman Coulter Personal Care Diagnostics, Palo Alto, California, U.S.A.). The samples for testing were collected as per the manufacturer's instructions for each test exactly as if the person had been defecating directly into the toilet bowl (EnterixOBT) or into a paper saddle (FlexSureOBT). In the EnterixOBT test, the sampling device is a brush (LiFung, Hong Kong) having a plastic stem or handle (approx. 185 mm length, 4-6 mm diameter) and flexible bristles (approx. 15 mm length). The sampling device for the FlexSureOBT test is a solid paddle or "popsicle" stick. To avoid bias, sampling for each test was standardised and blinded. For EnterixOBT, samples were collected by five brush strokes of the upright surface of the stool. Where loose stools were concerned, the brush was swirled around the stool five times. For FlexSureOBT, sampling was carried out as per manufacturer's instructions at random points on the stool.

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All tests were developed three-four days after sampling and all tests were read by two independent readers. The results are shown in Table 2 below.

### Results

5 TABLE 2 Test results for stripe-spiked stool samples.

	EnterixOBT (n=5)		FlexSureOBT (n=5)	
	Reader A	Reader B	Reader A	Reader B
Positive	5	5	1	1
Negative	0	0	4	4

### Discussion

Although the number of samples tested in this study is small, EnterixOBT appears to be able to detect a significant quantity of blood better than FlexSureOBT when the blood is striped along the surface of the stool. This difference is presumably due to the different methods of sampling employed by each test. As a result, EnterixOBT appears to have a clear advantage over FlexSureOBT in terms of the clinical detection of occult blood on faecal material, for example, in the detection of colorectal neoplasia.

Persons skilled in this art will appreciate that variations and modifications may be made to the invention as broadly described herein, other than those specifically described without departing from the spirit and scope of the invention. It is to be understood that this invention extends to include all such variations and modifications.

**CLAIMS:**

1. A method for collecting a sample derived from faecal material, comprising contacting the faecal material with a fluid and subsequently collecting a sample of the fluid with a brush or brush-like device having flexible or semi-flexible bristles, wherein the sample of the fluid is collected within the bristles of the brush or brush-like device.
2. A method according to claim 1, wherein the fluid is water.
3. A method according to claim 1 or claim 2, wherein the bristles of the brush or brush-like device have a length of from 0.2 to 3 cm, preferably from 1 to 2 cm.
4. A method according to claim 1, wherein the sample collected with the brush or brush-like device is transferred to an assay device for subsequent testing.
5. A method according to claim 4, wherein said assay device is a test device for detecting occult blood or one or more other indicators of a pathological condition in the faecal material from which the sample is derived.
6. A method for the detection of occult blood in faecal material, which comprises the steps of:
  - i. contacting the faecal material with water to disperse any blood present in or on the faecal material into the water,
  - ii. subsequently collecting a sample of the water with a brush or brush-like device having flexible or semi-flexible bristles, wherein the sample of the water is collected within the bristles of the brush or brush-like device; and
  - iii. detecting the presence of blood, if any, in the sample.

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7. A method according to claim 6, wherein the presence of blood, if any, in a sample is detected by means of a guaiac test.
8. A method according to claim 6, wherein the presence of blood, if any, in the sample is detected by means of an immunochromatographic test.
9. An assay kit for testing faecal material, which comprises a sample collection device which is a brush or brush-like device having flexible or semi-flexible bristles, together with means for detection of an analyte in a sample derived from the faecal material.
10. A kit according to claim 9, wherein the bristles of the brush or brush-like device have a length of from 0.2 to 3 cm, preferably from 1 to 2 cm.
11. A kit according to claim 9, wherein said means for detection is a test device for detecting occult blood or one or more other indicators of a pathological condition in the faecal material from which the sample is derived.

**Prior United States Application(s)**

I hereby claim the benefit under 35 U.S.C. §120 of any United States application(s), or §365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. §112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR §1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application:

Application Serial Number	Date of Filing (day, month, year)	Status - Patented, Pending, Abandoned
PCT/AU99/00310	27 April, 1999	Pending

As a named inventor, I hereby appoint the following registered practitioner to prosecute this application and to transact all business in the Patent and Trademark Office connected herewith:

Kevin M. Farrell, 35,505 (1)

All correspondence and telephone communications should be addressed to Kevin M. Farrell, Farrell & Associates, P.C., P.O. Box 999, York Harbor, ME 03911, telephone number (207) 363-0558, which is also the address and telephone number of each of the above listed attorneys.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

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x H.M. Chandler

Date: November 28, 2000

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**DECLARATION FOR PATENT APPLICATION**

As a named inventor, I hereby declare that:

My residence, post office address and citizenship is as stated below next to my name;

I believe that I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled: Sample collection method, the specification of which

☐ is attached hereto.

☒ was filed on 27 October 2000 as Application Serial Number \_\_\_\_\_ and was amended on \_\_\_\_\_ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a).

**Prior Foreign Application(s)**

I hereby claim foreign priority benefits under 35 U.S.C. §119(a)-(d) or §365(b) of any foreign application(s) for patent or inventor's certificate, or §365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or PCT International application having a filing date before that of the application on which priority is claimed:

Country	Application Number	Date of Filing (day, month, year)	Date of Issue (day, month, year)	Priority Claimed Under 35 U.S.C. 119
AU	PP3237	28 April 1998		Yes <input checked="" type="checkbox"/> No <input type="checkbox"/>
				Yes <input type="checkbox"/> No <input type="checkbox"/>

**Prior United States Provisional Application(s)**

I hereby claim the benefit under 35 U.S.C. §119(e) of any United States provisional application(s) listed below:

Application Number	Filing Date

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May 8, 2001

**FACSIMILE COVER SHEET**

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SUBJECT: U.S. Application No.: 09/763,154  
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Applicant(s): Howard Milne Chandler

Application No.: 09/763,154

Filing Date: October 27, 2000

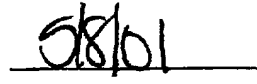
Title: SAMPLE COLLECTION METHOD

Docket No.: 0141-2004

## CERTIFICATE OF FACSIMILE TRANSMISSION

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FARRELL &amp; ASSOCIATES, P.C.



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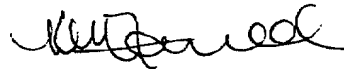
Sir:

On October 27, 2000, Applicant filed a U.S. National Stage Application claiming priority to International Application No. PCT/AU99/00310. In January of 2001, a Verified Statement Claiming Small Entity Status was filed as part of the above-referenced U.S. National Stage application. The Deposit Account Statement dated March 30, 2001, includes a charge for \$1,000 for the filing of the U.S. National Stage application.

-2-

Attached are copies of the executed Verified Statements, Transmittal Letter, and postcard receipt papers filed on January 5, 2001. The postcard receipt was stamped by the mail room at the U.S. Receiving Office. Applicant's Attorney hereby confirms that the attached copies are true copies of the originally mailed correspondence, returned postcard receipt, and official notation entered by the U.S. Receiving Office. Applicant's Attorney respectfully requests a reimbursement of \$500.00, one-half of the total fees paid on October 27, 2000, to be deposited to Deposit Account No. 06-0130.

Respectfully submitted,



Kevin M. Farrell  
Attorney for Applicant(s)  
Registration No. 35,505

York Harbor, ME

Dated: 5/8/01

0141\ARC\2004VS.TL

2004-05-08 12:53 PM

January 5, 2001

**THIS WILL ACKNOWLEDGE RECEIPT OF:**

Executed Verified Statements Claiming Small Entity Status, Transmittal Letter, and Request for Reimbursement (w/2 copies), with Certificate of Mailing.

Applicant(s): Howard Milne Chandler  
U.S. National Stage of PCT/AU99/00310  
International Filing Date: April 27, 1999  
Title: SAMPLE COLLECTION METHOD

Docket No.: 0141-2004

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0141-2004-456660

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Applicant(s): Howard Milne Chandler

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International Filing Date: April 27, 1999

Title: SAMPLE COLLECTION METHOD

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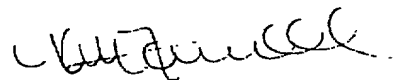
The Honorable Commissioner  
of Patents and Trademarks  
Washington, D.C. 20231

Sir:

Please file the enclosed Verified Statement Claiming Small Entity Status in the above-identified patent application.

Applicant's Attorney respectfully requests a reimbursement of \$500.00, one-half of the total fees paid on October 27, 2000, to be deposited in Deposit Account No. 06-0130. This request is made within the three-month period allowed for such reimbursement. Two duplicate copies are enclosed for accounting purposes.

Respectfully submitted,



Kevin M. Farrell  
Registration No. 35,505  
Attorney for Applicant(s)

York Harbor, ME 03911

Dated: 11/10/01

US - SMALL  
ENTITY

Applicant or Patentee: \_\_\_\_\_ Attorney's  
Serial or Patent No.: \_\_\_\_\_ Docket No.: \_\_\_\_\_  
Filed or Issued: \_\_\_\_\_  
For: \_\_\_\_\_

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY  
STATUS (37 CFR 1.9 (f) and 1.27 (b)) — INDEPENDENT INVENTOR

As a below named inventor, I hereby declare that I qualify as an independent inventor as defined in 37 CFR 1.9 (c) for purposes of paying reduced fees under section 41 (a) and (b) of Title 35, United States Code, to the Patent and Trademark Office with regard to the invention entitled Sample collection method described in

☐ the specification filed herewith  
☒ application serial no. \_\_\_\_\_, filed 27 October, 2000  
☐ patent no. \_\_\_\_\_, issued \_\_\_\_\_

I have not assigned, granted, conveyed or licensed and am under no obligation under contract or law to assign, grant, convey or license, any rights in the invention to any person who could not be classified as an independent inventor under 37 CFR 1.9 (c) if that person had made the invention, or to any concern which would not qualify as a small business concern under 37 CFR 1.9 (d) or a nonprofit organization under 37 CFR 1.9 (e).

Each person, concern or organization to which I have assigned, granted, conveyed, or licensed or am under an obligation under contract or law to assign, grant, convey, or license any rights in the invention is listed below:

☐ no such person, concern, or organization  
☒ persons, concerns or organizations listed below\*

\*NOTE: Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 CFR 1.27)

FULL NAME ENTERIX INC.  
ADDRESS 348 US Route One, Falmouth, Maine 04105 United States of America  
☐ INDIVIDUAL ☒ SMALL BUSINESS CONCERN ☐ NONPROFIT ORGANIZATION

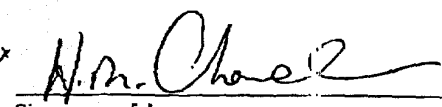
FULL NAME \_\_\_\_\_  
ADDRESS \_\_\_\_\_  
☐ INDIVIDUAL ☐ SMALL BUSINESS CONCERN ☐ NONPROFIT ORGANIZATION

FULL NAME \_\_\_\_\_  
ADDRESS \_\_\_\_\_  
☐ INDIVIDUAL ☐ SMALL BUSINESS CONCERN ☐ NONPROFIT ORGANIZATION

I acknowledge the duty to file in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28 (b))

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

Howard Milne CHANDLER

NAME OF INVENTOR	NAME OF INVENTOR	NAME OF INVENTOR
		
Signature of Inventor	Signature of Inventor	Signature of Inventor
<u>November 28, 2000</u>		
Date	Date	Date

U.S. Small  
Business

Applicant or Patentee: \_\_\_\_\_ Attorney's Dkt. No. \_\_\_\_\_  
 Serial or Patent No.: \_\_\_\_\_  
 Filed or Issued: \_\_\_\_\_  
 For: \_\_\_\_\_

**VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY  
 STATUS [37 CFR 1.27(c)] - SMALL BUSINESS CONCERN**

I hereby declare that I am

- ☐ the owner of the small business concern identified below:  
☒ an official of the small business concern empowered to act on behalf of the concern identified below:  
 NAME OF CONCERN ENTERIX INC.

ADDRESS OF CONCERN 348 US Route One, Falmouth, Maine 04105 United States of America

I hereby declare that the above identified small business concern qualifies as a small business concern as defined in 13 CFR 121.3-18, and reproduced in 37 CFR 1.9(d), for purposes of paying reduced fees under section 41(a) and (b) of Title 35, United States Code, in that the number of employees of the concern, including those of its affiliates, does not exceed 500 persons. For purposes of this statement (1) the number of employees of the business concern is the average over the previous fiscal year of the concern of the persons employed on a full-time, part-time or temporary basis during each of the pay periods of the fiscal year, and (2) concerns are affiliates of each other when either, directly or indirectly, one concern controls or has the power to control the other, or a third party or parties controls or has the power to control both.

I hereby declare that rights under contract or law have been conveyed to and remain with the small business concern identified above with regard to the invention, entitled:

Sample collection method

by inventors Howard Milne CHANDLER described in

- ☐ the specification filed herewith.  
☒ application Serial No. \_\_\_\_\_, filed 27 October, 2000  
☐ patent No. \_\_\_\_\_, issued \_\_\_\_\_

If the rights held by the above-identified small business concern are not exclusive, each individual, concern or organization having rights to the invention is listed below\* and no rights to the invention are held by any person, other than the inventor who could not qualify as an independent inventor under 37 CFR 1.9(c) if that person made the invention, or by any concern which would not qualify as a small business concern under 37 CFR 1.9(d), or a nonprofit organization under 37 CFR 1.9(e). \*NOTE Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities (37 CFR 1.27)

Name \_\_\_\_\_

Address \_\_\_\_\_

☐ Individual ☐ Small Business Concern ☐ Nonprofit Organization

Name \_\_\_\_\_

Address \_\_\_\_\_

☐ Individual ☐ Small Business Concern ☐ Nonprofit Organization

I acknowledge the duty to file, in this application of patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. [37 CFR 1.28(b)]

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING

TITLE OF PERSON OTHER THAN OWNER

ADDRESS OF PERSON SIGNING

SIGNATURE \* Howard Milne Chandler DATE 11/28/2000  
Chief Executive Officer  
\*357 Princes Point Rd, Falmouth, Maine, 04106 USA